EXPERIMENTAL MANIPULATION OF FOOD BODIES IN *Cecropia*

Heather L. Johnson

Biology Department
University of Utah
201 South Biology Building
Salt Lake City, Utah 84112

Dr. Diane W. Davidson
and Patricia Folgarait
University of Utah

Neotropical *Cecropia* trees range from Mexico to Argentina and are typical of disturbed habitats. *Cecropia* plants provide food and housing for the ants in return for known or presumed protection from herbivores and vine cover. Glycogen-rich Mullerian bodies (MB's) are the predominant food source for ants and are produced at sites called trichilia. Working with *Cecropia* (prov.) *"tessmannii",* we continuously removed MB's from 10 of 20 plants in a common garden and left the remaining control plants unmanipulated. Control plants produced fewer MB's over the lifespan of a trichilia. Therefore, plants appear to regulate MB production so that energy is not wasted in biotic defense if appropriate ants are not present.

INTRODUCTION

Cecropia are myrmecophytes, or plants with cavities or domatia which are regularly occupied by ants. In many myrmecophytes (ant-plants), including *Cecropia* (Schupp 1986), resident ant colonies protect their hosts from herbivores and/or encroaching vines. Dr. Davidson's *Cecropia* project, in conjunction with research done by P. Folgarait, investigates interspecific patterns in relative investment in biotic, chemical, physical, phenological and other defenses against herbivores. Despite having chemical and physical defenses (tannins, lignins, and fiber), myrmecophytic *Cecropia* rely substantially on resident ants as a form of "biotic defense."

The relative biotic investment for *Cecropia* plants is nutrition supplied for ants. *Cecropia* makes several forms of ant food, including parenchymal tissue on internal stem walls and lipid-rich pearl bodies beneath leaves. In the species we investigated, pearl bodies are few or absent after the development of other

myrmecophytic traits. Another food source, glycogen-rich Mullerian bodies (MB's) (Rickson 1971, 1976), represents the plant's principal investment in biotic defense. These MB's are produced at the base of each petiole, on small, brown, hairy platforms termed "trichilia".

Resource based theories of plant defense explain how plant resources are invested to maximize benefits and minimize costs of defense (e.g. McKey 1984; Coley *et al.* 1985). Because ant-related defenses must be synthesized continuously, they require constant investment by the plant. Therefore, biotic defenses are thought to be favored when leaves have comparatively short leaf lifetimes or rapid turnover. Chemical defenses have a high fabrication expense but relatively low costs for continuing upkeep. Chemical defenses are thought to be favored with increases in the lifespans of plant parts. Since plant resource environments are often correlated with leaf lifetimes (Coley *et al.* 1985; Bryant *et al.* 1985; Bazzaz *et al.* 1987), the leaf lifetime theory relates plant resource environments to relative investment in biotic and chemical defenses. In general, we would expect a positive correlation between resource availability and the ratio of investment in biotic versus chemical defenses.

There are several indications that chemical and biotic defenses of *Cecropia* could be costly. First, there is a negative correlation between tannin levels and leaf production rates in *Cecropia peltata* (Coley 1986). Secondly, when *Cecropia* are introduced to new environments, trichilia production is lost (Janzen 1973; Rickson 1977; Putz and Holbrook 1988). Thirdly, in interspecific comparisons, growth and defense investments tend to complement each other (Davidson era/. 1991). Thus, when comparisons are made between very close relatives, light-demanding species typical of large riverine disturbances grow more rapidly and acquire myrmecophytic traits later in development than do slow growing species of small forest light gaps. On average, the latter species also have longer leaf lifetimes and higher production of biotic defenses per unit leaf area (Davidson and Fisher 1991). With these indications that biotic defenses may be costly, we set out to determine whether or not *Cecropia* have mechanisms for regulating MB production in response to the presence or absence of symbiotic ants.

Our study focused on MB production in *C. "tessmannii"',* a small gap species from western Amazonia (Peru). (Presently considered a subspecies of riverine *Cecropia membranacea, Cecropia "tessmannii"* will probably soon be elevated to species rank and possibly given its local name *"pungara** [C.C. Berg, personal communication]). If the carbon investment in MB's is costly, and if ants are not present, plants might conserve resources and devote them either to growth or to other (e.g.

chemical) defenses. We manipulated the removal of MB's to test the following hypothesis: plants respond to the accumulation of food bodies (simulating absence of ants) by reducing the production of these ant rewards.

METHODOLOGY

Cecropia "tessmannii" seeds were put out for germination on March 5, 1991. Germinating seedlings were transplanted from sphagnum to soil on May 9, 1991, and transplanted to one gallon pots on June 10, 1991. On August 14, 1991, twenty *Cecropia "tessmannii"* plants were paired up with each other based on similarities of height and total number of leaves produced. One member of each pair was assigned to each treatment, i.e., to either the control or the experimental groups.

To simulate the absence of ants, the ten control plants were allowed to accumulate their MB's. Duct tape traps prevented loss of any fallen MB's and enabled us to associate fallen MB's with particular trichilia. We removed MB's from experimental plants every other day at approximately the same time of day (approximately 11 a.m.). For each plant, we initially monitored MB production in the two topmost trichilia, which were already producing MB's. These data were retained for comparative purposes, but were not used in the final analyses because we did not have measurements of total MB production over the entire lifetime of those trichilia. As new leaves were produced, we followed production by their trichilia, as well as keeping track of the birthdates of these leaves, and starting and ending dates of Mullerian body production. We recorded MB counts for a total of two new trichilia per plant and labelled them A and B (respectively, older and lower, and younger and higher) to permit comparisons between experimental and control plants. Leaves included in the study ranged in developmental order from the eleventh through the fourteenth leaves produced by individual plants.

The plants were rotated to different positions within the greenhouse every 6 days to allow for a comparable environment for all of the plants. Computer-controlled greenhouse bays on the University of Utah's Biology building rooftop maintained a constant hot, humid environment, and the plants were watered twice daily with distilled water. Mean 24 hour temperature and humidity were, respectively, 79.3°F and 51.5%.

For the experimental plants, MB counts were taken every other day starting August 14, 1991, and were continued until December 22, 1991, when all of the trichilia had completely stopped producing MB's. Once a lettered trichilium in an experimental plant stopped producing MB's, food bodies were also enumerated on like-lettered trichilia of control plants. By cutting out the

trichilium and magnifying it at 120x on a Wild Heerbrugg dissecting microscope, we could quantify the number of MB's remaining inside trichilia after we had ceased collecting MB's. Ninety percent of the control plant trichilia contained an additional 3-4 MB's, whereas those of experimental plants were empty. These additional Mullerian bodies were included in the final total count.

One major problem we encountered initially with all of the control plants was a build up of fungus on the most mature trichilia, below trichilia A and B. To curb this problem on control plants, we allowed approximately 150 Mullerian bodies to collect, before removing them. We stopped removing the MB's of control plants on September 9, 1991, when the problem ceased, and took final counts as prescribed above.

A Wilcoxon matched-pairs signed-ranks test was used to compare lifetime MB production by trichilia A and B in experimental and control plants.

RESULTS AND DISCUSSION

For individual control and experimental plants, Table 1 displays the total MB counts summed for trichilia A and B. In experimental plants, these totals represent production over the full lifetimes of the two trichilia. The medians for the two groups (control plants=1327 and experimental plants=1659) differed by 332 MB's. As predicted, MB production in experimental plants significantly exceeded that of control plants (£=0.0026 for a one-tailed test). The control plants may have diverted their carbon based resources to growth, or to chemical defenses. The difference discovered between experimental and control plants provides opportunities for further research. *C. "tessmannii's"* flexibility in response to the environment demonstrates its ability to change its production levels if MB's accumulate, as they would in the absence of ants.

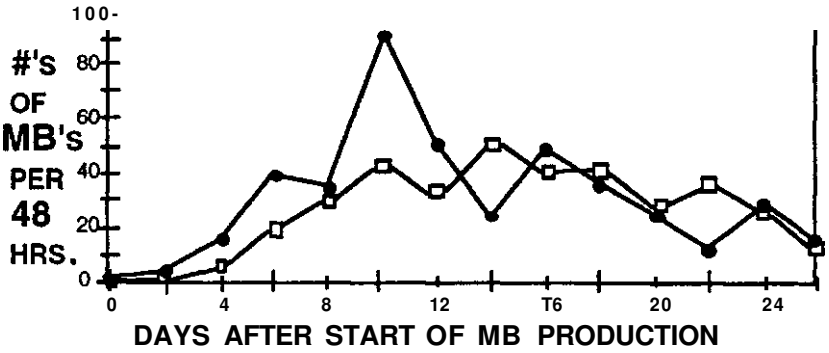
According to McKey's (1984) theory predicting relative investment in biotic versus chemical defenses, the cumulative costs of ant rewards are hypothesized to scale as a *linear* and increasing function of leaf age. However, we found that the rate of production of MB's decreases over time during a trichilia lifetime. For leaves A and B of both experimental and control plants, the average lifetime of a productive trichilia was 93.1 days. Through the course of this lifetime, MB production increased during the first 20-24 days. At that point, it began to decrease and to fluctuate among numbers lower than ten for another 30-100 days, until production ceased or the leaf fell. For two experimental plants, Figure 1 shows MB counts for the first twenty-six days. MB production by the A trichilium began on August 16, 1991, for plant #2E and on August 28, 1991, for plant #5E. Because

periods of MB production by the two trichilia overlapped only partially, it seems unlikely that weather trends account for the decline in MB productivity through time in each trichilia. Moreover, although space limitations preclude inclusion of data on all plants, the data in Figure 1 are generally representative of productivity trends with trichilia age. The observed decline in the level of protection afforded to older leaves may reflect the decreasing value of these leaves to the overall photosynthetic productivity of the plant.

Table 1. Total numbers of Mullerian bodies produced by trichilia A and B on the individual control and experimental plants over the total lifespans of trichilia.

PLANT NUMBERS	CONTROL PLANTS	EXPERIMENTAL PLANTS
#1	1380	1587
#2	1404	1735
#3	1509	1525
#4	1274	1643
#5	1032	1651
#6	1254	1811
#7	1736	1786
#8	1244	1667
#9	1050	1962
#10	1459	1588
Median	**1327**	**1659**

Figure 1. Total MB's produced per 48 hours during the first twenty-six days of production on trichilium A, for two experimental plants. See text for details.



a- **Plant #5E Trichilia A (#11) August 16 - September 14**
= Plant #2E Trichilia A (#13) August 28 - September 21

REFERENCES

Bazazz, F.A., Chiariello, N.R., Coley, P.D., and Pitelka, L.F. (1987). Allocating resources to reproduction and defense. *Biotropica,* 37: 58-67.

Bryant, J.P., Chapin, F.S., III, Reichardt, P., and Clausen, T.P. (1985). Adaptation to resource availability as a determinant of chemical defense strategies in woody plants. In *Chemically Mediated Interactions between Plants and Other Organisms. Advances in Phytochemistry* (ed. G.A. Cooper-Driver and T.Swain), 19:219-37.

Coley, P.D. (1986). Costs and benefits of defense by tannins in a neotropical tree. *Ecology,* 70:238-41.

Coley, P.D., Bryant, J.P., and Chapin, F.S., III. (1985). Resource availability and plant antiherbivore defense. *Science,* 230:895-9.

Davidson, D.W., Fisher, B. L. (1991). Symbiosis of ants with *Cecropia* as a function of light regime. In *Ant-Plant Interactions* (eds. C. Huxley & D. Cutler), Oxford University Press, Oxford, U.K., pp. 289-309.

Davidson, D.W., Foster, R.B., Snelling, R.R., Lozada, P.W. (1991). Variable composition of some tropical ant-plant symbioses. In *Plant-Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions,* (eds. P.W. Price, T.M. Lewinsohn, G.W. Fernandes, & W.W. Benson); John Wiley & Sons, Inc.

Janzen, D.H. (1969). Alleopathy by mymecrophytes: The ant *Azteca* as an allelopathic agent of *Cecropia. Ecology,* 50:147-153.

Janzen, D.H. (1973). Dissolution of mutualism between *Cecropia* and its *Azteca* ants. *Biotropica,* 5:15-28.

McKey, D. (1984). Interaction of the ant-plant *Leonardoxa africana* (Caesalpiniaceae) with its obligate inhabitants in a rainforest in Cameroon. *Biotropica,* 16:81-99.

Putz, F.W. and Holbrook, P.J. (1988). Further observations on the dissolution of mutualism between *Cecropia* and its ants: the Malaysian case. *Oikos,* 53:121-5.

Rickson, F.R. (1971). Review of glycogen plastid differentiation in Mullerian body cells of *Cecropia peltata. Annals New York Academy of Sciences,* 210:104-114.

Rickson, F.R. (1976). Anatomical development of the leaf trichilium and Mullerian bodies of *Cecropia peltata* L. *American Journal of Botany,* 65:1266-1271.

Rickson, F.R. (1977). Progressive loss of ant-related traits of *Cecropia peltata* on selected Caribbean islands. *American Journal of Botany,* 64:585-92.

Schupp, E.W. (1986). *Azteca* protection of *Cecropia:* ant occupation benefits juvenile trees. *Oecologia,* 70:379-385.